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FINAL REPORT

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Indianmeal Moth Granulovirus as an Alternative to Methyl Bromide for Protection of Dried Fruits and Nuts

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EXECUTIVE SUMMARY

The purpose if this research was to further expedite the commercialization of the granulovirus (GV) isolated from the Indianmeal moth (IMM), a cosmopolitan pest of stored products, including dried fruits and nuts. Production and formulation methods were developed in the early 1980s and the methodology was patented by the United States Department of Agriculture in 1991. The dust formulation of the GV is envisioned as a protectant against infestation by this insect to prevent economic damage for extended periods. In 1999 AgriVir LLC, obtained the patent rights and began producing the formulation in commercial quantities. In late 2001 and early 2002 the United States Environmental Protection Agency and California Environmental Protection Agency respectively registered the GV. AgriVir, LLC was the registrant. The trade name for the product is NutGuard-V / FruitGuard-V®, herein after referred to as AgriVir-GV.

Three objectives were identified in the project and included: 1) Reduce infestation of Indianmeal moth in dried fruits and in-shell or shelled nuts by topical or complete coverage application of AgriVir-GV; 2) Determine attractiveness of the formulation components as to IMM oviposition and larval survival; 3) Determine if five methyl bromide potential alternative fumigants (methyl iodide, phosphine, propylene oxide, carbonyl sulfide, and sulfuryl fluoride) inactivate the GV if fumigation was warranted.

Two types of commodity tests were used during these studies. Laboratory tests using glass jars maintained at 80±2°F or large-scale tests simulating bulk storage conditions in 32-gallon containers containing prunes, raisins, walnuts or pistachios. In laboratory assays the commercial products were found to be less potent compared to the ARS batch. In the commodity tests, jars and 32-gallon containers, the efficacy of AgriVir-GV was, for the most part, less than we had observed using our formulations. The reasons for the reduction in activity/efficacy are unknown but could be due to low active ingredient (virus capsules), nonviable virus capsules produced, partial inactivation due to high temperature in transit, or some other factors in production, harvesting, formulation, shipping and storage.

When mated female IMM were placed in an arena allowing them to choose between components of the GV formulation (i.e. wheat germ, wheat bran) vs. non-treated commodity females were capable of identifying formulation treated commodity. However, the threshold levels for discrimination were significantly higher than the amount of formulation used when treating the commodity. These data show that the levels applied commercially would not promote oviposition by mated females**.** Survival of larvae on commodities treated with formulation components without the GV was likewise not increased at the treatment levels recommended.

Results of the studies concerning inactivation of the GV showed that like methyl bromide, methyl iodide and propylene oxide also inactivates or kills GV. However, phosphine, carbonyl sulfide and sulfuryl fluoride did not impact the activity of the formulated GV. This provides industry with fumigants that could be used after application of AgriVir GV. The commodity could be subjected to carbonyl sulfide, phosphine, or sulfuryl fluoride to control IMM or other species of arthropod pests without inactivating the GV. Methyl bromide, methyl iodide and propylene oxide are not compatible with GV.

Introduction

The average annual production of dried fruits and nuts from California is over one million metric tons, and is worth nearly $1.5 billion U.S. (USDA 1997). Postharvest processing increases product value still further. Postharvest (storage) insects cause commodity losses during storage by direct damage, product contamination, and creation of favorable conditions for mold growth and product degradation. Costs to the dried fruit and nut industry due to insect-related product loss and control measures are substantial. The dried fruit and tree nut industry depends on multiple fumigations with methyl bromide (MB) or phosphine (PH_3) for postharvest insect control. Repeated infestations may occur in storage. There is no protection for these commodities once they have entered marketing channels. Indianmeal moth (IMM), *Plodia interpunctella* (Hübner) causes the majority of damage to dried fruits and nuts. Besides direct damage, copious amounts of silk may render the commodity unmarketable. The control method of choice is fumigation (MB or PH_3).

In 1992 MB was designated an ozone depleter (UNEP 1992). Under the provisions of the U.S. Clean Air Act (USEPA 1993), use of MB has been severely restricted and maybe eliminated for all but a few uses. More recent regulations by the U.S. have exempted quarantine and preshipment treatments.

The granulovirus (GV) infectious to IMM (IMM-GV), a member of the Baculoviridae, was first isolated and characterized in 1968 by Arnott and Smith. Studies by Hunter et al. (1973) demonstrated efficacy against IMM infestations in raisins, almonds and walnuts. Cowan et al. (1986) developed a production/formulation method for IMM-GV that was later patented (Vail 1991). Other investigators confirmed high levels of efficacy on various dried fruit and nuts (Hunter et al. 1977, 1979) and grains (McGaughey 1975a; Kinsinger and McGaughey 1976). Control ranged from 77 – 100%. Over 95% control was reported in most cases and reached 100% in a number of cases (see Relevant Literature).

In late 2001 and early 2002 the commercial production formulation of IMM-GV, Nutguard-V/Fruitguard-V®, was registered respectively by the USA-EPA and California-EPA for use as a protectant. The registrant is AgriVir, LLC, Washintgton, D.C. NutGuard-V/FruitGruard-V® is exempt from tolerance and labeling requirements. This product (AgriVir-GV) was used in these studies along with a batch produced at the USDA, ARS laboratory, Parlier, CA that was used as the standard for comparison (ARS-26).

An integrated system utilizing controlled atmospheres (CA) and GV was reported by Johnson et al. (1998 and 2002) to provide short and long-term control of IMM infesting walnuts, almonds, or raisins. Application of the GV after an initial CA disinfestation treatment controlled the infesting population of IMM and prevented damage by IMM even when the commodity was exposed to populations far higher than would be found in any commercial storage facility. Results of these large-scale tests were very encouraging. Several meetings have been held with the dried fruit and nut industry to determine how, when, and where the GV might be applied to provide long-term protection. We later tested persistence of GV on treated nuts over a period of 2 years at 80°F. These studies showed continued efficacy of the GV over the entire 2 years. Moth emergence was reduced by more than 90% through the test period with both raisins and almonds under extremely high infestation pressure.

The project expands our efficacy base for the GV as a protectant that when applied properly reduces the number of fumigations needed to control IMM. Rapid control (fumigants) of insects is required upon delivery to prevent damage and prevent infestation of processing and storage facilities. The GV would reduce the number of fumigation treatments significantly and provide economic control of IMM. The GV also provides a mechanism to protect commodities in marketing channels and until consumption. AgriVir GV is exempt from tolerance and labeling requirements. Many of these commodities may be stored in warehouses where they become re-infested by IMM. The GV provides a mechanism to protect commodities that is not currently available to the dried fruit and nut industry.

The research will reduce the dependency on the use of fumigants and maintain quality of the product. We estimate the cost of a GV application to be close to that of MB. However, the more fumigations that are eliminated, the more cost effective the GV becomes; some commodities maybe fumigated 5-10 times. If used properly, quality of the product will be maintained in storage and in marketing channels and reduce overall use of current or future fumigants.

Successful integration of the GV in the current storage and marketing channels would impact all areas of California where these commodities are processed or stored. There are approximately 80 dried fruit and nut handlers in California. Commodities to be tested include walnuts, pistachios, raisins, and prunes. All studies were conducted in cooperation with San Joaquin Valley industries.

The studies were specifically conducted to further define and examine the potency of the commercial formulations and also determine the impact of currently used fumigants, and the impact of storage conditions (i.e. high temperatures) on activity and persistence of the GV. We also measured the impact of commercial rates of current and potential fumigants on activity. We also investigated the ability of the commercial formulation GV to provide adequate control of Indianmeal moth larvae in semi commercial tests conducted with walnuts, pistachios, raisins and prunes. Damage can be reduced by both methods but level of efficacy may be commodity dependent and depend on the interstitial spaces in the commodities (i.e. raisins vs. walnuts). The size of the interstitial spaces may determine how far down the infestation penetrates. We also investigated if the components (all organic) were attractive for oviposition by female IMM at the treatment levels recommended. The impact of the complete formulation components as well as individual components was also investigated.

Results and Discussion

OBJECTIVE I: Determine potency of the early, commercially-produced batches of AgriVir-GV and the effectiveness of different methods of application of the GV on dried fruits and nuts.

<u>Task 1.1: Relative potency of early batches of commercially produced AgriVir-GV compared to a standard ARS formulation as determined by laboratory bioassay.</u>

Relative potency of the first three commercially produced batches of AgriVir-GV is shown in Table 1 (AgriVir-A, -B and -C). A batch produced in the laboratory by USDA, ARS, is also included and was used as the standard for comparison (ARS-26). Notice there are two designations for Batch C (C1 and C2), which represent the first and second shipment, respectively, received over time from AgriVir, LLC and used in different tests as described and reported herein (Task 1.2 and 1.3, respectively).

The active ingredient of AgriVir-GV is the granulovirus capsule. Potency of the product is based on the number and viability of the GV capsules present in the formulated powder. The commercially produced batches were 20 to 129 times less potent than the ARS standard (Table 1). Batch C1, which was assayed at the same time as Batches A and B, was only 20 times less potent than ARS-26 and had the highest potency of the three batches at the time. Batches A, B, and C1 are the batches included in Task1.2 below.

The second shipment of Batch C, Batch C2, had lost much of its original potency and was found to be 883 times less potent than ARS-26 (Table 1). We suspect that this shipment was likely exposed to high temperatures for some undetermined period of time while in transit, which adversely affected viability of the virus capsules. Unfortunately, Batch C2 was the batch used in our large-scale commodity tests, Task 1.3, and the low potency was not discovered until after the tests were conducted. Further discussion will follow below.

<u>Task 1.2: Laboratory tests to determine the effectiveness and longevity of AgriVir-GV, Batches A, B, and C, compared to the ARS-26 standard as a protectant of shelled almonds.</u>

The recommended label rate for NutGuard/FruitGuard is from 1 to 5 oz. per ton (31 to 156 mg/kg). In laboratory tests, we tested the first three batches of AgriVir-GV and included an ARS batch (ARS-26) for comparison. Rates of 2.5 or 5 oz per ton (78 or 156 mg/kg) were tested. Almonds (500 g) were spread onto porcelain trays in a single layer, one tray for each test material and dosage (untreated and water treated controls were also included). Batch C2 had not yet been received and was not included in these assays. The formulated GV powder from each batch was suspended in distilled water and 15 ml of the GV suspension was applied to 500 g of almonds. The almonds were allowed to air dry (15 to 30 minutes) and then placed inside sterilized 2-quart jars and closed using filter paper lids. The following day, each jar was infested with 4 mg of IMM eggs (n = 200 to 220). The jars were held at 27°C and 55% RH for 35 days to allow the simulated IMM infestation to develop before being evaluated for insect survival and damage to the almonds.

After the 35-day storage period, the tests were evaluated to determine the relative effectiveness of each batch of GV in protecting the almonds from IMM by counting and recording the number of live IMM larvae, pupae, and adults and by counting and recording the number of damaged nuts (none or minor, moderate, and severe). The reduction of IMM population and reduction of damage due to IMM was calculated from levels found in the control groups. The IMM and damaged nuts were discarded and the remaining nuts were returned to the jar and the lid replaced. The jars were frozen for 24 hours to kill any live insects that may have been missed and then returned to the simulated long-term storage conditions. The infestation and evaluation of the tests was repeated every 3 months to determine long-term efficacy and persistence of AgriVir-GV stored at 27°C and 55% RH.

Results showing survival of IMM, damage caused by IMM, and the percentage of reduction (protection) of these parameters by the GV protectant are shown in Table 2 for up to 9 months storage. Reduction of the pest population continued to be excellent (99 to

100%) even after 9 months and at both dosages tested, regardless of the batch of GV (Table 2). Protection of the almonds also remained high throughout the test period as reflected in the percent reduction of damage caused by IMM. At a dosage rate of 5oz/ton (156 mg/kg), Batch C1 continued to protect the almonds from insect damage by 97 to 100% for up to 9 months. This was comparable to the ARS-26 batch, even though Batch C1 had potency 20 times less than ARS-26. Level of protect (reduction in damage) declined to less than acceptable levels for Batches A and B, falling below 90% after 6 and 9 months storage (Table 2). These batches were from 59 to 129 times less potent than ARS-26, respectively.

<u>Task 1.3: Large-scale commodity tests to determine effectiveness of different methods of application of the GV protectant on dried fruits and inshell nuts against IMM in storage.</u>

Four different methods of application of AgriVir-GV microbial pesticide were tested. Descriptions are given in Table 3. Since we will be using acronyms to refer to the treatments throughout this report, it is important that the methods associated with the abbreviations used be clear to the reader (Table 3). Unprocessed inshell walnuts, raisins, prunes, and inshell pistachios were used in the study. The aim of this test was to determine if results comparable to bulk treatments of the entire commodity could be obtained if only the top portion (12” depth) of the load was treated with GV. AgriVir-C2 was used in these tests. AgriVir-C2 was applied either suspended in water (aqueous applications) or as a dry powder or dust mixed with ground wheat germ as a carrier (dry application). The GV was applied, (1) as an aqueous treatment over a shaker as the commodity was being fed into a bin and all of the commodity was treated (AQ-Bulk); (2) as an aqueous treatment as described above, but with only the top 12 inches of the commodity receiving the GV treatment (AQ-Top); (3) as an aqueous treatment applied via a backpack sprayer and only to the surface of the commodity already being stored in the bin or can (AQ-Surface); and (4) the GV dust or powder mixed with ground wheat germ and applied to the commodity by tumbling them together in a large cement mixer for 10 minutes and also just the top 12 inches of the commodity treated (GVWG-Top). Refer to Table 3. An untreated control group was included for each commodity for comparison.

The different GV treatments of each commodity were set up in 32-gallon plastic garbage cans to a depth of 24 inches to simulate the depth of short bins (4’ W x 4’ D x 2’ H) commonly used by industry. The entire commodity (bulk), the top half (top), or the surface only (surface) was treated for each commodity tested. The ‘top’ or ‘surface’ treated cans were filled with 12 inches of treated commodity over 12 inches of untreated commodity beneath. All treatments were replicated four times for a total of 20 cans per commodity. The cans were placed inside holding rooms using a Latin-square design. The surface of the commodity inside each can was seeded with IMM eggs: 30 mg (1,500 – 1,650 eggs) on raisins or prunes; or 20 mg (1,000 – 1,100 eggs) on walnuts or pistachios. The cans were closed with fiberglass screen held in place with the plastic lids, which had been converted into ‘rings’ by cutting out the centers. Storage conditions in the rooms were maintained at 27°C and ambient humidity. Walnuts and pistachios were stored for 6 weeks and raisins and prunes for 8 weeks to allow the infesting population of IMM time to become adults.

Damage caused by the infesting population of IMM was also assessed at the end of the test period. Only prunes (dried fruit) and pistachios (nuts) were included in damage assessments. Walnuts were not evaluated, because the quality of the test material used was extremely substandard and quality assessments could not be made. Damage to raisins was not evaluated due to time constraints and lack of resources, because so many units (berries) are contained in a sample. At the end of the test, 2 kg samples of prunes and pistachios were taken from both the upper and lower layers of each can. Damage was assessed based on effect on marketability of individual units, i.e., nuts (pistachio; 500 g subsample; n = 350-375) or dried fruits (prune; 1 kg subsample; n = 160-175). We used four categories of damage or effect on marketability: (1) none, (2) minor (pinhole) damage with little or no affect; (3) moderate damage resulting in downgraded marketability, and (4) severely damaged units, including any presence of larvae, pupae or pupal cases on the unit, resulting in an unmarketable unit. Other non-related types of damage were ignored.

Survivability of the test populations was evaluated by separating the developed IMM from the commodity. The entire contents of each can was passed twice through a shaker with an expanded metal insert to allow smaller objects (insects) to fall to the bottom, thereby separating the insects from the fruits and nuts. The number of IMM larvae, pupae, and adults present in each can were collected, counted and recorded.

IMM survival after different methods of application is shown in Table 4. Historically, an effective commodity treatment would provide at least 90 to 95% control of the targeted pest population. Most of the treatments with AgriVir-C2 resulted in only moderate reductions of the infesting IMM population, controlling IMM by 65 to 93%. Best results were obtained with AgriVir-C2 on walnuts, reducing IMM populations by 82 to 93%. Mixed results were obtained for prune and pistachio. The dust application (GVWG-Top) failed when applied to prunes, but the other methods of application provided from 66 to 82% control. The backpack, surface applied treatment (AQ-Surface) failed on pistachios, but good results were obtained from the other methods showing 79 to 88% control of IMM on pistachios. In general, except for the obvious failures noted above, the application of GV to just the top portion (12”) of stored products controlled IMM as well as if the entire commodity (bulk) had been treated with the microbial pesticide.

Damage to prunes and pistachios are shown in Table 5a and 5b. Application of AgriVir-C2 to prunes resulted in 47 to 77% reductions in damage by IMM, except for the failed GVWG-Top application. Damage was reduced in pistachios from 31 to 88%, but both the AQ-Top and AQ-Surface treatments failed with pistachio (Table 5a). These damage figures correlate with survival data shown in Table 4 for the same treatments. Table 5b repeats some of the data presented in 5a, but is rearranged to more clearly show the comparisons of results between upper and lower layers. Treatments applied to prunes provided moderate, yet comparable, protection to both upper and lower layers for all methods of application, except GVWG-Top. Although two aqueous treatments failed to protect the upper layer of pistachios, all of the treatments protected the lower layer by reducing damage to the untreated portion from 51 to 88% (Table 5b).

The application of AgriVir-GV either in bulk or to the top 12” of bulk-stored dried fruits and nuts successfully decreased infesting populations of IMM, in most cases (Table 4). However, the success of the microbial pesticide to protect the commodity from damage from IMM was less demonstrable (Table 5a and 5b). We generally consider an effective commodity treatment will provide control of the pest population from 90 to 95% or above. The lack of potency (non-viability of GV capsules) of AgriVir-C2 used in this test was inadequate to provide effective protection of the commodity. However, as AgriVir, LLC continues to improve their production and QC departments, NutGuard-V/FruitGuard-V

(AgriVir-GV) will be worthy of use to protect stored products from invasive populations of IMM either as a stand alone protectant or as a tool in a pest management strategy tailored to meet the challenges facing the agricultural community.

OBJECTIVE II: Determine attractiveness of different components of the GV formulation to ovipositing adults of IMM on selected commodities and survival of IMM on GV formulation with or with wheat germ additive.

Task 2.1: Effect of different components of the GV formulation on ovipositional preference of IMM.

A 24 x 18 inch sleeve cage was used as the arena for testing ovipositional preference of adult IMM to different components of the patented GV formulation along with the wheat bran diet on which they are normally reared and to the ground wheat germ used as a carrier when the formulation is applied as a dust. The different components tested are shown in Table 6. In order to properly evaluate the test based on survival of progeny resulting from the ovipositing adults, and since it was not our intent to kill the insects in this test series, we tested the different components of the GV formulation without the virus present.

The grid below is a diagrammatic representation of the positions within the sleeve cage that preliminary tests found to be unbiased. No positional effects were observed using this system.

1	4	5
2	3	6

The initial series of tests consisted of three containers each of two components placed randomly in the cage. Each combination of components was replicated three times. Test containers consisted of open 8 oz paper cups holding a thin layer of test material one of which was placed in the center of each position on the grid. Test insects were six mated pairs of IMM adults. These were introduced into the cage that was then covered with black fabric to eliminate light bias. After 72hr the adults were removed and 20 grams of rearing diet was added to the cups that were then sealed and incubated at 27°C until adults emerged.

Table 7 summarizes results of this series of tests. Statistical analysis showed that adults were attracted to the rearing diet and wheat germ carrier more than to the formulation components. This indicates that the addition of formulation alone (such as in an aqueous suspension) to a commodity should not make it more susceptible to IMM infestation as long as the virus remains infectious.

Task 2.2: Effect of different components of the GV formulation on ovipositional preference of IMM in the presence or absence of dried fruit or nuts.

A subsequent series of trials examined oviposition preference for treated versus untreated commodity. These tests were designed to determine if use of the GV product as a dry dust application, with ground wheat germ added as a carrier to distribute the GV, affected the ovipositional preference of female IMM. Treated commodity received a combination dose of the B and D components shown in Table 6. A dosage of 90 µg/g of the B component plus a dosage of 0.1% by weight concentration of wheat germ carrier (D component) was applied to the treated commodity and is based on dosages used in IPM trials on walnuts reported by Johnson et al (1998). Specifics on commodities and additives are given in the following table.

Commodities	Total treated (g)	B component (g)	D component (g)	Aliquots
Inshell walnuts	700	0.063	0.637	4 nuts
Almond meats	900	0.081	0.819	30 g
Processed raisins	1500	0.135	1.365	30 g
Walnut meats	700	0.063	0.637	30 g
Inshell almonds	850	0.0765	0.7735	25 g

Commodity was placed in a glass gallon jar with the respective amounts of additives and manually tumbled until it appeared to be uniformly coated. For each test replication three aliquots of commodity were removed from the treatment jar and put into 8 oz paper cups that, along with three untreated aliquots, were randomly placed in the sleeve cage arena described above. Handling of test insects, exposure time, addition of rearing diet, and incubation period until adult emergence were as described above. Tests of each commodity were replicated five times.

Table 8 summarizes results from all of these trials. Statistically there was no difference between treated and untreated almond meats, processed raisins, walnut meats, or inshell almonds. However, inshell walnuts treated with GV formulation and wheat germ were significantly more attractive than untreated walnuts. This may be explained by the possibility of a food source immediately available to the hatching IMM larva on almonds, almond shells, walnut meats, and raisins, but not available to the feeding larva on the shell of the walnuts. Therefore, the wheat germ additive coating the walnut shell provided a food source and attracted the larvae to feed, increasing the survival rate of the insect. This supports the philosophy behind using wheat germ as a carrier of the virus in order to stimulate feeding that the larva may ingest a lethal dose of virus before damaging the commodity being protected.

Task 2.3: Effect of components of GV formulation on survival of IMM larvae.

Numerous attempts to directly feed components of the GV formulation to IMM larvae resulted in no survivors from components B or C (Table 6). The only materials that produced adult survivors were the larval diet (A) and the ground wheat germ carrier (D). Therefore, tests were designed to determine if use of the GV product as a dry dust application, with ground wheat germ as the carrier agent, affected the feeding and survivability of IMM. If so, the presence of wheat germ would stimulate feeding and, in the presence of viable GV, would serve to enhance the effectiveness of the treatment in protecting the commodity. However, if for some reason, the GV capsules were to become inactivated, the presence of wheat germ may actually increase survival of IMM, thereby increasing the susceptibility of the commodity to IMM infestation, which would be counterproductive to our goal. To gallon jars containing 800 g of inshell walnuts we added formulation component B at 90µg/g in addition to the wheat germ (D) at four concentrations of 1.0%, 0.5%, 0.1%, and 0% by weight. Jars were tumbled on a rotary tumbler for 20 minutes at 40 rpms. We also included an untreated control. Three jars per treatment were used in each of two replications. Twenty milligrams of IMM eggs were added to each jar and the test was incubated at 27°C until adult emergence. After the first generation of survivors emerged and were counted the jars were frozen to stop all insect activity. To evaluate insect damage 25 nuts were randomly picked out of each jar, cracked and examined closely.

Although statistics showed that only the 1.0% level of wheat germ was significantly different from the control, both survivors and % nut damage increased as the concentration of wheat germ increased. Figure 1 represents the combined results of both replications of this test.

OBJECTIVE III: Determine effects of selected fumigants on the activity of GV.

We exposed GV (ARS-26) to six different fumigants. The fumigants tested were methyl bromide (MB), phosphine (PH3), methyl iodide (MI), propylene oxide (PPO), carbonyl sulfide (CS), and sulfuryl fluoride (SF) (Table 9). We know that MB inactivates GV and that any MB treatment must be conducted prior to the application of GV. We also know that PH3 has no effect on GV and therefore commodities treated with GV are compatible with PH3 fumigation. GV was exposed to the selected fumigants at typical dosage rates and times of exposure. All fumigations were conducted at 27°C and normal atmospheric pressure (NAP), except for the PPO treatment, which was under vacuum (27 inches of mercury; VAC). After fumigation, the GV was assayed using discriminating dosages (10 or 1 mg/kg) to determine if there was any affect on the viability of the virus capsules. Untreated controls and GV controls were included in the test for comparison.

Fumigants inactivating GV included MB. MI and PPO. Fumigants having no effect on the viability of GV were PH3, SF, and CS and would be compatible with the application of GV as a protectant (Table 9).

Summary and Conclusions

There is no evidence that IMM adults are attracted to any of the actual components of the GV formulation for oviposition, probably because none of these components support survival of larvae. However, when the formulation is mixed with a ground wheat germ carrier, as with dry applications of the product, the wheat germ attracts ovipositing females and also increases survival of IMM. As long as the GV is infectious this may actually enhance it efficiency as the wheat germ acts to stimulate feeding and ingestion of the GV capsules. On the other hand, if the virus becomes inactivated the presence of wheat germ may actually encourage and support the IMM infestation of the treated commodity. For this reason, it is advisable to avoid areas where exposure to high temperature may occur in order to assure that viability and longevity of the virus is not adversely affected.

Small-scale tests in the laboratory, simulating long-term storage of almonds, showed that GV batches (AgriVir-C1) having as much as 20 times less potency than the standard ARS batch continued to provide excellent protection in reducing both IMM populations and damage to the commodity for up to 9 months.

Results from the large-scale tests conducted at the USDA ARS research facility in Parlier, CA were below expectations due to the very low potency of AgriVir-C2 (883 times less) that was provided for these tests. In spite of unacceptable potency, we still obtained significant reductions in IMM population and damage for most of the application methods tested. However, survival and damage was greater than would be acceptable to industry. Best results would be obtained by treating the entire commodity (AQ-Bulk) with the GV protectant for the time being, until AgriVir can improve production and quality control issues.

The GV-based protectant, AgriVir-GV, is a microbial pesticide and its efficacy is, by its very nature, dependent upon the number and viability of the GV capsules present (active ingredient). Virus capsules are heat labile and a proportion continually loses viability over time. As this occurs, the effectiveness of the pesticide degrades over time. Hence, the approach to long-term protection of stored products is to apply a very high dosage of a highly potent batch of GV to begin with. Then, in spite of gradual degradation, the level of control and protection remains well above an acceptable threshold of control (90 to 95% or higher) for an extended period of time (6 to 12 months or longer). If you look at Table 1, a dosage of 156 mg/kg (the upper label dose for AgriVir-GV) is within the upper confidence limits and well above the LD_{95} for the standard batch (ARS-26). That is why a GV batch with potency comparable to ARS-26 has been shown to provide excellent protection of stored products from IMM infestations in large-scale tests for extended periods of time (Johnson et al. 1998, Johnson et al. 2002). However, the estimated LD_{95} values for all of the commercially produced batches tested (Table 1) are all well above the recommended label rate of 156 mg/kg, particularly for Batch C2. This is why we are seeing inadequate protection of the commodities in our large-scale tests even with “time zero” infestations of IMM.

We are confident in the technology of microbial pesticides and the use of insect viruses to protect stored products from arthropod pests. It is our opinion that as AgriVir, LLC improves its production and quality control departments the potency and standardization of subsequent batches of AgriVir-GV will continue to improve. When they achieve levels of potency comparable to the ARS standard, they will be able to provide and market a product worthy of consideration by the agricultural community as a very effective tool in the battle to protect food and fiber from agricultural pests, such as the Indianmeal moth.

Appendix A: Objective I Tables

Table 1. Estimated lethal dosages (LD_{50} and LD_{95}) and relative potency of GV batches.

IMM-GV Batch	Estimated LD (95% confidence limits) LD_{50} (mg/kg)	LD_{95} (mg/kg)	Relative Potency [1]
ARS-26	0.24 (0.02 – 0.73)	8.4 (2.2 – 365.1)	—
AgriVir-A	14.1 (9.9 – 20.0)	730 (408 – 1,521)	59 times less
AgriVir-B	30.9 (8.9 – 131.3)	3,087 (496 – 183,590)	129 times less
AgriVir-C1 [2]	4.9 (2.3 – 9.3)	1,916 (680 – 8,584)	20 times less
AgriVir-C2 [3]	212 (93 – 560)	1.09×10^6 (nr)	883 times less

[1] Potency is relative to ARS-26 batch and is based on comparison of LD_{50} values.
[2] AgriVir-C1 was the first shipment of AgriVir Batch C and was the batch used in the small jar tests with almonds meats (Objective I, Task 1.2).
[3] AgriVir-C2 was the second shipment of AgriVir Batch C and was the batch used in the large-scale commodity tests with unprocessed raisins, prunes, pistachios, and walnuts (Objective I, Task 1.3).

Table 2: Comparison of effectiveness and persistence of commercial and laboratory produced batches of IMM-GV applied to almond meats in the laboratory. [1]

Time at 27°C (Months)	GV dosage (oz./ton)	GV Batch	Survival of IMM		Damage Due to IMM	
			% Survival (mean ± SD)	% Reduction	% Damage (mean ± SD)	% Reduction
0	0	Control	84.2 ± 12.6	—	71.0 ± 6.4	—
	2.5	AgriVir-A	0.6 ± 0.1	99.4	9.5 ± 4.6	86.7
	2.5	AgriVir-B	0.4 ± 0.4	99.6	11.0 ± 5.8	84.6
	2.5	AgriVir-C	0.2 ± 0.2	99.9	3.9 ± 3.4	94.5
	2.5	ARS-26	0	100	0.2 ± 0.4	99.8
	5.0	AgriVir-A	0.4 ± 0.2	99.5	6.0 ± 5.5	91.6
	5.0	AgriVir-B	0.2 ± 0.2	99.9	5.6 ± 4.1	92.2
	5.0	AgriVir-C	0	100	1.0 ± 1.5	98.6
	5.0	ARS-26	0	100	01 ± 0.1	100
3	0	Control	85.8 ± 5.8	—	71.6 ± 1.0	—
	2.5	AgriVir-A	0.6 ± 0.5	99.3	5.0 ± 2.6	93.1
	2.5	AgriVir-B	0.4 ± 0.4	99.6	7.6 ± 5.5	89.4
	2.5	AgriVir-C	0	100	2.0 ± 2.5	97.3
	2.5	ARS-26	0	100	1.4 ± 1.6	98.2
	5.0	AgriVir-A	0.2 ± 0.2	99.8	3.4 ± 3.6	95.3
	5.0	AgriVir-B	0.2 ± 0.2	99.8	2.8 ± 1.4	96.1
	5.0	AgriVir-C	0	100	0.4 ± 0.5	99.5
	5.0	ARS-26	0	100	0.4 ± 0.5	99.5

Table 2 (cont.): Comparison of effectiveness and persistence of commercial and laboratory produced batches of IMM-GV applied to almond meats in the laboratory. [1]

Time at 27°C (Months)	GV dosage (oz./ton)	GV Batch	Survival of IMM: % Survival (mean ± SD)	Survival of IMM: % Reduction	Damage Due to IMM: % Damage (mean ± SD)	Damage Due to IMM: % Reduction
6	0	Control	84.5 ± 9.2	—	81.6 ± 4.3	—
	2.5	AgriVir-A	0.4 ± 0.6	99.6	7.3 ± 3.0	91.0
	2.5	AgriVir-B	0.3 ± 0.4	99.7	8.3 ± 2.1	89.7
	2.5	AgriVir-C	0.2 ±0.2	99.9	2.6 ±1.3	96.8
	2.5	ARS-26	0.2 ± 0.2	99.9	0.7 ± 0.7	99.2
	5.0	AgriVir-A	0.3 ± 0.2	99.7	5.2 ± 2.5	93.6
	5.0	AgriVir-B	0.2 ± 0.2	99.9	4.2 ± 1.5	94.8
	5.0	AgriVir-C	0	100	01 ± 0.1	100
	5.0	ARS-26	0	100	0.2 ± 0.4	99.8
9	0	Control	86.6 ± 4.5	—	77.4 ± 6.5	—
	2.5	AgriVir-A	0.6 ± 0.5	99.3	9.8 ± 5.9	87.4
	2.5	AgriVir-B	0	100	9.4 ± 2.4	87.9
	2.5	AgriVir-C	0	100	7.3 ± 3.4	90.6
	2.5	ARS-26	0	100	1.3 ± 1.0	98.3
	5.0	AgriVir-A	0.2 ± 0.2	99.8	7.9 ± 6.0	89.8
	5.0	AgriVir-B	0.2 ± 0.2	99.9	9.3 ± 6.8	87.9
	5.0	AgriVir-C	0	100	2.0 ± 2.6	97.5
	5.0	ARS-26	0	100	1.0 ± 1.6	98.7

[1] Tests were conducted in the laboratory in two-quart jars under constant temperature and humidity control (27°C and 55% RH). Tests were replicated two times with three jars per dose and GV batch. Tests will continue up to a storage time of 12 months.

Table 3. Description and shorthand designations of the different methods of application of AgriVir-GV (NutGuard/FruitGuard®) used in large-scale tests with selected stored products. [1]

Method (designation)	Description [2]
Aqueous treatment, Whole bin treated (AQ-Bulk)	NutGuard/FruitGuard, suspended in water (aqueous treatment or AQ), was applied using No. 8006 *cone-jet nozzles* mounted over the commodity as it moved along a shaker and into the bin. Dose was based on the weight of the entire commodity in the can. All of the commodity in the can was treated with the GV protectant (Bulk).
Aqueous treatment, Top half treated (AQ-Top)	NutGuard/FruitGuard, suspended in water (aqueous treatment or AQ), was applied using No. 8006 *cone-jet nozzles* mounted over the commodity as it moved along a shaker and into the bin. Dose was based on the weight of the top 12 inches of commodity. Only the top half (12 inches) of the commodity was treated (Top).
Aqueous treatment, Surface layer treated (AQ-Surface)	NutGuard/FruitGuard, suspended in water (aqueous treatment or AQ), was applied using a *backpack sprayer* to the surface of the commodity already in the bin. Dose was based on the weight of the top 12 inches of commodity. Only the surface of the commodity was sprayed and then left undisturbed (Surface).
Dry dust treatment, Top half treated (GVWG-Top)	NutGuard/FruitGuard (GV), mixed with ground wheat germ (WG) (60 mesh; 0.1% by wt), was applied as a dust formulation (dry treatment or GVWG). Dose was based on the weight of the top 12 inches of commodity. The dry powder was applied by tumbling the GVWG and the commodity together for 10 minutes using a large cement mixer then pouring an amount representing the top 12 inches into the bin (Top).
Untreated control (Control)	An equivalent amount of commodity, not treated with the GV protectant, but stored in the same containers and in like manner.

[1] Dosage of NutGuard/FruitGuard was 5 oz/ton (156 mg/kg) in all tests.
[2] All commodities were received in short bins (4'W x 4'D x 2'H) and handled in short bins if aqueous treated using nozzles and the shaker. Space limitations required that all commodities be tested in 32-gallon plastic garbage cans filled to a depth of 24" to simulate 2-foot high bins. All treatments were replicated four times for a total of 20 cans per commodity.

Table 4. IMM SURVIVAL: Comparison of effectiveness of AgriVir-GV protectant when applied to selected commodities by different methods.

Commodity	Method of GV Application	Survival of IMM (mean ± SD)		
		Percentage survival	% Reduction [1]	Number per KG
Raisin	Control	13.0 ± 7.5	—	3.9 ± 2.2
	AQ-Bulk	3.1 ± 1.2	76.2	0.9 ± 0.4
	AQ-Top	4.2 ± 1.2	67.7	1.3 ± 0.4
	AQ-Surface	4.4 ± 0.9	66.2	1.3 ± 0.3
	GVWG-Top	3.2 ± 0.8	75.4	1.0 ± 0.3
Prune	Control	12.8 ± 1.5	—	3.5 ± 0.4
	AQ-Bulk	3.4 ± 1.4	73.4	0.9 ± 0.4
	AQ-Top	2.3 ± 1.3	82.0	0.6 ± 0.4
	AQ-Surface	4.4 ± 2.6	65.6	1.2 ± 0.1
	GVWG-Top	41.1 ± 9.6	0	11.3 ± 2.7
Pistachio	Control	39.2 ± 5.0	—	8.6 ± 1.1
	AQ-Bulk	6.6 ± 1.1	83.2	1.4 ± 0.2
	AQ-Top	8.0 ± 1.7	79.6	1.8 ± 0.4
	AQ-Surface	31.6 ± 3.5	0	7.0 ± 0.8
	GVWG-Top	4.8 ± 0.8	87.8	1.0 ± 0.2
Walnut	Control	8.8 ± 0.9	—	3.2 ± 0.3
	AQ-Bulk	1.6 ± 0.6	81.8	0.6 ± 0.2
	AQ-Top	0.7 ± 0.2	92.0	0.3 ± 0.1
	AQ-Surface	0.9 ± 0.5	89.8	0.3 ± 0.2
	GVWG-Top	0.6 ± 0.4	93.2	0.2 ± 0.1

[1] % Reduction of surviving IMM = ((% in control – % in treated)/% in control)*100.

Table 5a. IMM DAMAGE: Comparison of effectiveness of AgriVir-GV protectant when applied to selected commodities by different methods.

Sample	Method of	Percent damage due to IMM (mean ± SD)					
Layer	Application	None	Minor	Moderate	Severe	Total [1]	% Redx. [2]
				~ PRUNE ~			
Upper	Control	7.7 ± 3.3	64.0 ± 3.4	18.2 ± 3.9	9.9 ± 2.3	28.1 ± 3.4	—
	AQ-Bulk	70.8 ± 3.3	18.6 ± 4.5	3.1 ± 1.7	4.3 ± 1.7	7.5 ± 2.4	73.3
	AQ-Top	71.1 ± 7.9	17.8 ± 3.5	3.0 ± 2.1	5.0 ± 3.2	8.0 ± 5.3	71.5
	AQ-Surface	72.1 ± 5.7	18.6 ± 4.7	3.6 ± 0.8	3.8 ± 1.7	7.4 ± 1.1	73.7
	GVWG-Top	35.3 ± 10.3	36.7 ± 4.1	18.0 ± 5.2	6.6 ± 3.9	24.6 ± 5.4	12.5
Lower	Control	11.4 ± 6.1	71.1 ± 3.7	13.2 ± 2.7	2.9 ± 1.8	16.1 ± 2.6	—
	AQ-Bulk	83.0 ± 3.6	8.0 ± 1.8	4.6 ± 1.1	1.2 ± 0.5	5.8 ± 1.0	64.0
	AQ-Top	73.4 ± 10.3	13.9 ± 9.7	6.8 ± 3.2	1.7 ± 0.9	8.5 3.9±	47.2
	AQ-Surface	77.5 ± 7.5	16.4 ± 6.8	3.6 ± 2.5	0.2 ± 0.3	3.7 ± 2.2	77.0
	GVWG-Top	53.3 ± 9.0	24.5 ± 1.9	16.6 ± 7.7	3.5 ± 1.6	20.1 ± 8.5	0
				~ PISTACHIO ~			
Upper	Control	52.4 ± 3.9	19.0 ± 2.0	6.6 ± 1.8	7.4 ± 1.3	14.0 ± 0.9	—
	AQ-Bulk	5.3 ± 6.4	20.8 ± 4.3	7.8 ± 8.3	1.7 ± 0.5	9.6 ± 8.5	31.4
	AQ-Top	47.5 ± 8.6	19.9 ± 2.8	11.4 ± 8.4	2.8 ± 1.6	14.1 ± 9.9	0
	AQ-Surface	46.6 ± 3.1	18.4 ± 6.7	10.3 ± 4.5	7.6 ± 3.0	17.9 ± 5.8	0
	GVWG-Top	62.5 ± 10.6	12.0 ± 4.4	5.8 ± 1.9	1.2 ± 0.8	6.9 ± 2.4	50.7
Lower	Control	66.4 ± 2.6	14.2 ± 1.6	3.8 ± 1.6	1.1 ± 0.6	4.9 ± 2.1	—
	AQ-Bulk	68.0 ± 5.4	11.7 ± 4.2	2.8 ± 3.2	0.2 ± 0.2	2.9 ± 3.3	40.8
	AQ-Top	80.7 ± 2.2	4.2 ± 0.7	0.4 ± 0.2	0.1 ± 0.1	0.6 ± 0.1	87.7
	AQ-Surface	69.2 ± 4.3	12.0 ± 3.4	1.5 ± 0.8	0.9 ± 0.4	2.4 ± 0.7	51.0
	GVWG-Top	76.3 ± 5.6	6.0 ± 2.1	0.7 ± 0.8	0.2 ± 0.2	0.9 ± 0.8	81.6

[1] Total = Moderate + Severe pooled.
[2] % Redx. = % reduction in damage due to IMM = ((% in control – % in treated)/% in control) x 100.

Table 5b. IMM DAMAGE: Comparison of effectiveness of AgriVir-GV protectant when applied to selected commodities by different methods.

Commodity	Method of Application	Layer	Mean (± SD) IMM damage: Percentage [1]	% Reduction [2]	Number per KG
Prune	Control	Upper	28.1 ± 3.4	—	45.2 ± 4.9
		Lower	16.1 ± 2.6	—	26.0 ± 4.2
	AQ-Bulk	Upper	7.5 ± 2.4	73.3	12.5 ± 4.2
		Lower	5.8 ± 1.0	64.0	9.5 ± 1.7
	AQ-Top	Upper	8.0 ± 5.3	71.5	13.2 ± 8.5
		Lower	8.5 ± 3.9	47.2	13.8 ± 6.4
	AQ-Surface	Upper	7.4 ± 1.1	73.7	12.0 ± 1.8
		Lower	3.7 ± 2.2	77.0	6.0 ± 3.6
	GVWG-Top	Upper	24.6 ± 5.4	12.5	40.2 ± 8.5
		Lower	20.1 ± 8.5	0	33.0 ± 14.4
Pistachio	Control	Upper	14.0 ± 0.9	—	117.5 ± 7.2
		Lower	4.9 ± 2.1	—	41.0 ± 18.1
	AQ-Bulk	Upper	9.6 ± 8.5	31.4	77.0 ± 66.6
		Lower	2.9 ± 3.3	40.8	25.0 ± 28.2
	AQ-Top	Upper	14.1 ± 9.9	0	120.0 ± 84.7
		Lower	0.6 ± 0.1	87.8	4.5 ± 1.0
	AQ-Surface	Upper	17.9 ± 5.8	0	149.5 ± 49.7
		Lower	2.4 ± 0.7	51.0	19.5 ± 7.4
	GVWG-Top	Upper	6.9 ± 2.4	50.7	57.5 ± 19.5
		Lower	0.9 ± 0.8	81.6	7.5 ± 7.0

[1] Percentage damaged represents pooled moderate and severe damaged commodity.
[2] % Reduction in damage due to IMM = ((% in control – % in treated)/% in control) x 100.

Appendix B: Objective II Tables and Graphs

Table 6. Description of different components of the GV protectant formulation offered as choices to IMM adult females in test arena.

Tests	Letter Designation of Component	Description of Component
Oviposition preference	A	Larval rearing bran diet (Tebbets et al, 1978)
	B	Formulation diet (Vail, 1991) with uninfected larvae
	C	Formulation diet without larvae
	D	Ground wheat germ carrier

Table 7. Preference of IMM to different components of the GV protectant formulation as shown by the number of progeny from ovipositing females in choice experiments.

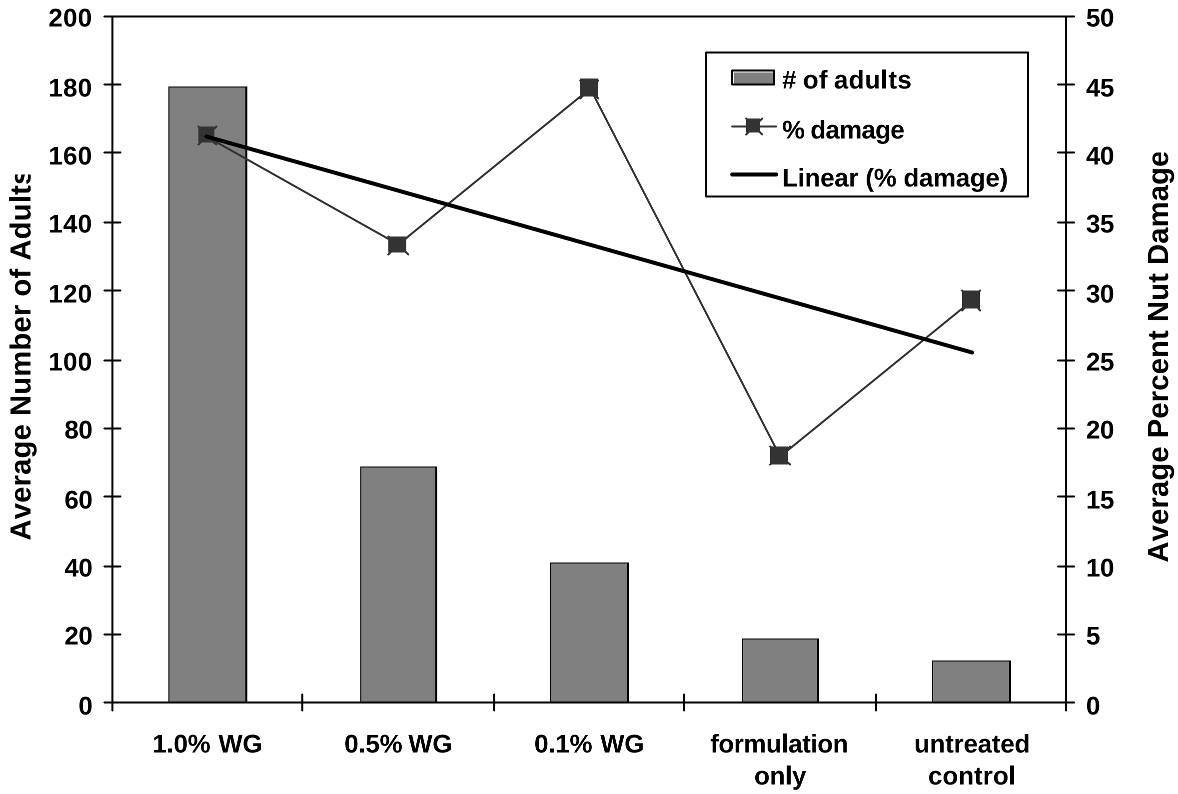
Pairing of Components (Choice)	Component	Number of progeny Total	Average ± SD
A v B	A	2,202	244.7 ± 16.0
	B	106	11.8 ± 7.3
A v C	A	2,266	251.8 ± 40.6
	C	193	21.4 ± 21.7
A v D	A	1,693	188.1 ± 41.0
	D	1,313	145.9 ± 43.3
B v C	B	262	29.1 ± 24.1
	C	529	58.8 ± 31.3
B v D	B	156	17.3 ± 17.3
	D	1,941	215.7 ± 31.7
C v D	C	163	18.1 ± 12.6
	D	2,046	227.3 ± 27.6
Pooled (choice interactions disregarded)	A	6,161	228.2 ± 32.5
	B	524	19.4 ± 16.2
	C	885	32.8 ± 21.9
	D	5,300	196.3 ± 34.2

Table 8. Results of oviposition preference trials on treated versus untreated commodities. [1]

Commodity	Mean # Adult survivors	95% Confidence Limits Lower	Upper
Inshell walnuts:			
Treated	124.67	112.557	139.977
Untreated	52.20	49.65	55.02
Almond meats:			
Treated	197.00	134.306	259.694
Untreated	151.67	88.972	214.361
Processed raisins:			
Treated	137.80	114.991	160.609
Untreated	139.267	116.457	162.076
Walnut meats:			
Treated	111.733		
Untreated	119.286		
Inshell almonds:			
Treated	144.58		
Untreated	126.67		

[1] "Treated" commodity received GV formulation and wheat germ as described in the text.

Figure 1. Effect of wheat germ (WG) additive on IMM survival and damage caused by IMM.



Appendix C: Objective III Tables

Table 9: Effect of selected fumigants on the activity (potency) of IMM-GV as determined by bioassay at discriminating dosages of the GV following exposure to the fumigants. [1]

Fumigant (treatment)	GV dosage (µg/g)	% Mortality	GV Inactivated?
Untreated control (no GV; no fumigant)	0	18.0	—
GV control (no fumigant)	10	98.7	—
	1	88.7	
Methyl Bromide ($56\ g/m^3$ for 24 hr)	10	0	YES
	1	0	
Methyl Iodide ($56\ g/m^3$ for 24 hr)	10	0	YES
	1	0	
Propylene Oxide ($96\ g/m^3$ for 24 hr)	10	12.8	YES
	1	11.5	
Phosphine (30 ppm for 5 days)	10	100	NO
	1	91.9	
Sulfuryl Flouride ($16\ g/m^3$ for 24 hr)	10	100	NO
	1	91.0	
Carbonyl Sulfide ($56\ g/m^3$ for 24 hr)	10	100	NO
	1	91.0	

[1] All fumigations performed at 70°F and normal atmospheric pressure, except propylene oxide, which was treated under vacuum.

Appendix D: References

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